The NuoEF subcomplex was crystallized and the structure was resolved at 1.7 Å and 1.9 Å when soaked with substrates. It is attempted to crystallize the other preparations containing subcomplexes in order to understand the structure and function of complex I.

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The role of Ndufaf2 in the assembly of mitochondrial complex I

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Mitochondrial complex I (NADH: ubiquinone oxidoreductase) is the first and largest enzyme complex in the mitochondrial respiratory chain. It is the major entry point to oxidative phosphorylation, catalysing the transfer of electrons from NADH to coenzyme Q, which is coupled to pumping protons out of the mitochondrial matrix. It plays a central role in mitochondrial metabolism, as nearly 50% of oxidative phosphorylation disorders result from a complex I deficiency. In humans, complex I is composed of 45 subunits, with a total molecular mass of 980 kDa. It has been shown that the assembly of complex I follows a semi-sequential order and requires a number of assembly factors, with defects of these assembly factors responsible for complex I deficiency. Ndufaf2 is an assembly factor involved in the late stages of complex I assembly. However, its actual function is still unclear. We have identified three patients with complex I deficiency harbouring mutations in *NDUFAF2*. The loss of Ndufaf2 in these patients results in reduced levels of mature complex I, with the subsequent accumulation of membrane arm subunits in a stalled ~600 kDa complex. The steady-state levels of the nDNA-encoded subunits, NDUFA9 and NDUFS2, are also affected. However, the translation of complex I mtDNA-encoded subunits is normal. Additionally, the assembly factor Ndufaf4 is up-regulated, with its accumulation in an ~150 kDa complex with the complex I subunit NDUFS3. These studies will help us to solve the puzzle of complex I assembly and to understand the function of Ndufaf2 in the assembly process.

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Semiquinone intermediates formed during catalysis by complex I

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Understanding quinone reduction by mitochondrial complex I remains a challenge due to limited structural and mechanistic data. Ubisemiquinones are considered intermediates of mitochondrial complex I catalysis on the basis of electron paramagnetic resonance studies on isolated protein, proteoliposomes and native membranes, however the number of species and their location remain poorly defined. Up to three species have been associated with complex I: SQ_{Nf}, SQ_{Ns} and SQ_{Nx}. The extremely slow relaxing specimen, SQ_{Nx}, is no longer considered relevant. The fast relaxing specimen, SQ_{Nf}, has never been observed in isolated complex I or tightly coupled proteoliposomes, so its association with complex I remains questionable. Finally, SQ_{Ns}, the slow relaxing radical, while well documented to be originating from complex I, has not been located in the structure. Some studies employ also hydrophilic quinone analogues that can react with the flavin site of complex I and form intermediates not specific to energy-transducing quinone reduction. Here, we revisit the semiquinone formation in complex I under physiologically relevant conditions, to further elucidate the mechanism of quinone reduction.

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Site-directed mutagenesis of residues involved in proton translocation by *Escherichia coli* complex I

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NADH:ubiquinone oxidoreductase (complex I) is the first and largest enzyme of mitochondrial respiratory chain. Mutations in the hydrophobic part of the enzyme have been linked to many neurodegenerative diseases. Complex I catalyses the transfer of two electrons from NADH to a quinone molecule coupled to the translocation of protons, resulting in the establishment of a proton motive force. The enzyme is present in a wide variety of species and is well conserved in the core subunits. The enzyme from *Escherichia coli* is an excellent model for the eukaryotic complex I and a recent crystal structure of the membrane domain of *E. coli* complex I heralds a significant breakthrough. However, the detailed mechanism of the action of complex I is still largely unknown. Mechanism of proton translocation through channels in the membrane is widely disputed, as well as the nature and extent of the conformational changes in the protein upon proton pumping. Several residues in antiporter-like subunits are predicted from the membrane domain structure to be important for proton translocation. This study focuses on the site-directed mutagenesis of those residues in order to elucidate the mechanism of proton translocation in *E. coli* complex I.

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A combined electrochemical, Resonance Raman and Far Infrared study on the respiratory complex I

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The NADH:ubiquinone oxidoreductase (complex I) is the largest energy transducing complex known so far. Complex I is a membrane protein formed by a membrane part, that is responsible for the proton pumping, and a soluble part that bears the redox cofactors. The X-ray crystal structure of the protein shows nine cofactors, one FMN and eight Fe-S clusters. Electrons enter via the FMN and flow through the Fe-S clusters towards the interface with the membrane part where the quinone reduction occurs. The coupling of the electron transfer and proton pumping across the membrane is one of the crucial questions that still need to be solved.

We used Resonance Raman (RR) spectroscopy to identify the signature given by the iron sulfur clusters in function of the redox state. The possibility to study the environment of transition metal in proteins derives from enhancement, sensitivity and selectivity for vibrations closely associated with atoms at the absorbing center(s) in the molecule. We combined the RR technique with an electrochemical cell and compared the reduction to the data obtained by reduction with NADH. Finally far infrared spectroscopy was used to obtain additional information on the cofactors and the coupling to proton translocation.

The RR data reveals specific signatures for complex I, a three-subunit and a soluble fragment corresponding to the peripheral domain, allowing the differentiation of contributions of the cofactors. Isotope labeling was introduced to the Fe–S centers in order to identify the Fe–S vibrations and at lower frequencies the signals (250–400 cm^{-1}) could be attributed to iron–sulfur stretching frequencies shifting in function of redox state and the specific environment. The influence of NADH on the spectral features will be presented and discussed.

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Structure of mitochondrial complex I

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Proton pumping NADH: ubiquinone oxidoreductase (mitochondrial complex I) from the aerobic yeast *Yarrowia lipolytica* is a very large membrane protein comprising 14 central and at least 28 accessory subunits [1]. We have crystallized complete complex I for X-ray crystallography [2]. The marked spatial separation of the ubiquinone reduction site near iron–sulfur cluster N2 from the proton translocation sites in the membrane arm strongly favours a conformational coupling mechanism. Energy conversion by complex I is discussed in the light of refined structural evidence.

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Iron sulfur clusters in complex I of *Yarrowia lipolytica* characterized by cw- and pulsed EPR

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Eight iron–sulfur clusters were detected by X-ray crystallography in complex I (NADH: ubiquinone oxidoreductase) of *Yarrowia lipolytica* [1]. However, in cw-EPR only five signals (N1 to N5) were observed. "EPR invisibility" of the remaining clusters may result from very fast relaxation of reduced paramagnetic states or a very negative midpoint potential keeping the clusters oxidized and diamagnetic, even in presence of reducing substrate.

The redox behavior of iron–sulfur clusters was analyzed by EPR monitored redox titrations of the isolated enzyme. In bovine complex I the binuclear cluster of the 75 kDa subunit has an extremely low potential and a "rollercoaster-like" profile of the midpoint potentials of iron sulfur clusters was described [2]. In contrast, the midpoint potentials of the EPR detectable clusters in *Y. lipolytica* complex I are all in the range between −300 and −200 mV, with the exception of the high potential cluster in the PSST subunit (N2).

Exchanging the histidine ligand of one of the clusters in the 75-kDa subunit to alanine did not result in changes or even loss of EPR signals upon reduction with NADH [3]. However, in the oxidized state a signal typical for a three iron four sulfur cluster appeared. If in the wild type enzyme reduced histidine ligated cluster was a very fast relaxing species, it should affect the relaxation of its next neighbors. We thus analyzed the relaxation of the clusters in wild type and mutant complex I by pulsed EPR techniques. The relaxation of the nearest cluster in the TYKY subunit (N4) was not affected by "turning off" the influence of the formerly histidine ligated cluster in the mutant enzyme. The T2 sensitive pulsed field sweep spectrum of complex I provides an insight into relaxation behavior of the involved paramagnetic centers. The spectrum was fitted using a model where the observable clusters N1-N4 were only affected by the fast relaxing cluster N5.

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